Nicotine: A Double-Edged Sword in Atherosclerotic Disease

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Chronic cigarette smoking is well-known to damage vascular endothelium, which initiates atherosclerosis by first manifesting as endothelial dysfunction and later progressing to cardiovascular diseases (CVD). Nicotine, a major component of tobacco smoke, is traditionally thought to be responsible for increased cardiovascular events through stimulation of the sympathetic nervous system, increased myocardial metabolic demand, impaired lipid metabolism, and activated platelet function. However, recent studies have demonstrated that nicotine, at lower doses, may be beneficial to the cardiovascular system. With binding to specific nicotinic acetylcholine receptors, nicotine can induce migration and proliferation of vascular cells, and hence enhances angiogenesis. Therefore, these seemingly inconsistent properties of nicotine may in fact give rise to novel and efficacious management strategies of CVD.

INTRODUCTION

Atherosclerosis is thought be to a multi-factorial disease of the arterial wall, which results from the interaction between circulating blood and mural cells. Therefore, pro-atherogenic molecules transported in the blood have the potential to induce atherosclerosis. Among the risk factors of atherosclerosis, smoking is strongly associated with the initiation and development of atherosclerotic diseases. Cigarette smoke contains more than 4,000 chemical constituents, of which nicotine is a major component. Epidemiological studies have also reported that the dose of nicotine consumption is proportional to the risk of cardiovascular diseases (CVD) related to atherosclerosis.[1] Nicotine has been shown to attract inflammatory cells onto endothelium and induce endothelial dysfunction,[2] which is an early marker of atherosclerosis.[3] However, in recent years, parallel studies have demonstrated that activation of nicotinic acetylcholine receptors (nAChRs), by nicotine-augmented proliferation[4] and migration[5] of endothelial cells, has the capacity to enhance angiogenesis.[6] Other reports have shown that such activation also exhibited properties that countered inflammation[7] and apoptosis.[8] Therefore, there is a growing body of evidence suggesting that nicotine is not absolutely hazardous to the vascular wall. This article reviewed the contradictory effects of nicotine.

EPIDEMIOLOGY OF CIGARETTE SMOKING AND CVD

Cigarette smoking is associated with a two to four-fold risk of CVD, and was perhaps the single most important of the preventable causes of cardiovascular morbidity and mortality, including coronary heart disease (CHD), stroke, aortic aneurysm, and peripheral

vascular disease.[9] According to a 2009 Work Health Organization report, 'currently, tobacco use kills 5.4 million people a year-an average of one person every six seconds- and accounts for one in 10 adult deaths world-wide'. Similarly, cigarette smoking had been a major health issue in Taiwan, contributing to 13.9% of the total mortality for men and 3.3% for women in the year 1994.[10] In a study evaluating acute myocardial infarction in Taiwan's young population from 2005-2008, cigarette smoking was shown to be the most prevalent risk factor.[11] Yusuf et al. indentified the risk of acute myocardial infarction associated with current tobacco smoking in the overall population (odds ratio 2.87 for current vs. never smoked).[12] Cessation of smoking could decrease the risk of CVD and cardiovascular death. A cohort study demonstrated that cessation of smoking in patients with CHD reduced the relative risk of death by 36% and that of myocardial re-infarction by 32%, compared with those who continued to smoke.[13]

CARDIOVASCULAR EFFECTS OF NICOTINE

Since nicotine is a major component of tobacco, the cardiovascular effects of nicotine have been previously studied in an effort to better quantify its systemic impact. Initially, the adverse effects of nicotine were emphasized, as listed in Table 1. Nicotine stimulated the sympathetic nervous system and resulted in increases of heart rate (increment, up to 10 to 15 beats/min) and blood pressure (5 to 10 mmHg). As a consequence, cardiac output and myocardial oxygen consumption were increased.[14] Kaijser et al. indentified that nicotine has a biphasic effect on coronary blood flow. First, nicotine could lead to increased myocardial metabolic demand and augment blood flow of large coronary vessels, but then decrease blood flow through alpha-adrenergic vasoconstriction of coronary resistance vessels. It was also determined that the increase of coronary blood flow after pacing was blunted in healthy nonsmokers chewing 4 mg of nicotine gum. This finding showed that nicotine could constrict coronary arteries in humans even at low doses.[15] Regarding nicotine's effect on lipid metabolism, there are conflicting data. Elevated low-density lipoprotein-cholesterol (LDL-C), decreased HDL-C, and acceleration of atherosclerosis were observed in

squirrel monkeys given oral nicotine with 6 mg/kg/day.[16] However, no changes in plasma concentrations of triglycerides, total cholesterol, LDL-C, HDL-C, or apolipoprotein A1 and B were found in healthy nonsmokers taking nicotine chewing gum (2 mg for each of eight times per day) for 2 weeks.[17] Fisher et al. demonstrated no progression of atherosclerosis in animals given a lower dose of nicotine (1 mg/kg per day) plus a 2% cholesterol diet.[18]

Research on platelet function in human smokers also yielded conflicting results. Some studies showed an increased propensity of thrombosis through such mechanisms as: (1) nicotine induced vessel narrowing with resulting turbulent flow that activated platelets to release adenosine diphosphate and in turn enhanced platelet aggregation;[19] (2) nicotine stimulated epinephrine release which promoted platelet aggregation and thrombus formation by α-adrenergic mechanism.[20] However, low dose nicotine gum (2 mg) or transdermal nicotine (Nicoderm, 21 mg/day) did not exhibit platelet activation.[21,22] An animal study revealed no increased platelet activity in rodents given nicotine over a long-term period of time.[23] However, these different effects were recently identified to be dose-dependent.[21-23]

EFFECTS OF NICOTINE ON NON-NEURONAL CELLS

The average serum concentration of nicotine in a daily-intake cigarette smoker was 0.31 μM (3.1×10^{-7} M),[24] with a peak serum level achieved within 10 minutes of smoking, and a corresponding half-life of approximately 2 hours.[25] Similar to platelet function studies, the effects of nicotine on non-neuronal cells, including angiogenesis, migration, proliferation, regulation of nAChRs, and cell cytotoxicity, was associated with its

Table 1. Cardiovascular adverse effects of nicotine

Increased in heart rate, myocardial contractility, cardiac output, and coronary blood flow[26]
Cutaneous and coronary vasoconstriction[27]
Endothelial injury and intimal hyperplasia[28]
Increased serum catecholamines[20]
Dyslipidemia: increased LDL, decreased HDL[16]
Platelets activation and thrombosis[19]

HDL, high-density lipoprotein; LDL, low-density lipoprotein.

concentration. Villablanca et al. provided the first evidence of nicotine-induced calf pulmonary artery epithelial cell proliferation in vitro. In that study, cell proliferation could be induced at concentrations lower than 10^{-8} M, but higher concentrations (> 10^{-6} M) resulted in cytotoxicity.[4] Thereafter, Park et al. reported that, in human umbilical vein endothelial cells (HUVECs), an amount of 10^{-10} to 10^{-6} M nicotine actually enhanced proliferation, whereas it inhibited proliferation and induced cytotoxicity at 10^{-4} M.[29] Human embryonic stem cells (hESCs) were first described in 1998,[30] and hESC derivatives had potential clinical and therapeutic applications.[31] Jin Yu et al. demonstrated that nicotine in fact enhanced hESCs-derived endothelial cell survival. In an animal model of ischemic heart (mice with ligation of the left anterior descending coronary artery), short-term (72 hours) exposure to nicotine (10^{-8} to 10^{-6} M) resulted in neovasculature formation. Their conclusion was that a low concentration of nicotine (10^{-8} M) could enhance hESCs-derived epithelial cell angiogenesis and thereby prevent apoptosis in hypoxemic condition via mitogen-activated protein kinases (MAPK) and AKT signal pathways.[32] Furthermore, nicotine was reported to increase smooth muscle cells (SMCs) proliferation and migration[33] under the stimulation of growth factors such as basic fibroblast growth factor (bFGF), platelet-derived growth factor (PDGF), and transforming growth factor (TGF) β1.[34] Nicotine was shown to inhibit SMCs apoptosis at concentrations of both 6×10^{-6} M and 6×10^{-8} M.[35] Nicotine could act on its nAChRs in vascular SMCs to activate the extracellular-signal-regulated kinase (ERK)/early growth response 1 gene (Egr-1) signal pathways that enhanced cell proliferation and neointimal formation after injury of arteries.[36] Such effects of nicotine on vascular SMCs are helpful to post-injury vascular repair and stabilization of atheromatous plaques. To conclude, significant cell death occurred at higher nicotine concentrations of 10^{-4} to 10^{-2} M, whereas increased proliferation, anti-apoptosis, and angiogenesis were observed at the lower concentrations of 10^{-8} to 10^{-6} M.[32] Table 2 summarizes the biological effects induced by nicotine at different ranges of concentration on non-neuronal cells.

ROLE OF nAChRs IN NICOTINE'S BIOLOGICAL EFFECTS

nAChRs are acetylcholine-gated ion channels consisting of homo- or hertero-pentamers, having a molecular mass of 290 kDa, arranged symmetrically with a central ionic pore, which are permeable to cations such

Table 2. Dose-dependent effect of nicotine on non-neuronal cells

Reference	Cell type	Dose of nicotine (M)	Duration of nicotine treated (hrs)	Biological effects
Dasgupta, 2009[37]	NSCLC Human breast cancer	10^{-6} to 10^{-7}	24	Angiogenesis; migration; invasion
Dom, 2011[38]	HRMECs	10^{-7}	-	Angiogenesis
Heeschen, 2002[39]	HUVECs	10^{-10} to 10^{-6}	12	Angiogensis; nAChR up-regulation
Park, 2008[29]	HUVECs	10^{-10} to 10^{-6}	48	Angiogenesis; mroliferation; migration
Villablanca, 1998[4]	Calf pulmonary artery epithelial cell	10^{-14} to 10^{-8}	36	Angiogenesis; proliferation
Lane, 2005[40]	Cervical cancer cell	6.2×10^{-8}	24	Angiogenesis; proliferation
Ng, 2007[41]	HMVECs	10^{-8}	24	Migration
Yu, 2009[32]	hESC-ECs	10^{-8}	48	Angiogenesis; nAChR up-regulation
Cucina, 2008[35]	SMCs	6×10^{-6} and 6×10^{-8}	24 and 72	Proliferation
Park, 2008[29]	HUVECs	10^{-4}	48	Cytotoxicity
Villablanca, 1998[4]	Calf pulmonary artery epithelial cell	> 10^{-6}	36	Cytotoxicity

hESC-ECs, human embryonic stem cell-derived epithelial cells; HRMECs, human retinal microvascular endothelial cells; HUVECs, human umbilical vein epithelial cells; nAChR, nicotinic acetylcholine receptors; NSCLC, non-small cell lung cancer cells; SMCs, smooth muscle cells.

as Na^+, K^+, and Ca^{2+}.[42,43] The homomeric α7-nAChR differed from the heteromeric nAChRs in that it was preferentially permeable to Ca^{2+} ions, rather than Na^+.[44] The biological significance of this preference is that the stimulation by nicotine induced a large increase in intracellular calcium.[44] A particular group of circulating stem cells, called endothelial progenitor cells (EPCs), was thought to participate in endothelial cell regeneration and neovascularization. Non-neuronal cells in the vascular wall, such as endothelial cells and SMCs, also express nAChRs. The findings and effects of nAChRs activation in EPCs and non-neuronal cells related to cardiovascular research included the following: (1) α7-nAChRs were one of the most plentiful nAChRs in epithelial cells;[45] (2) the addition of nicotine to endothelial cells (10^{-10} M, 12 hours) or exposure to hypoxia for 4 hours enhanced up-regulation of α7-nAChRs;[46] (3) increased expression of α7-nAChRs was found in the ischemic hindlimb of the mouse subject to femoral artery ligation;[46] (4) both mecamylamine and α-bungarotoxin were specific inhibitors of nAChRs (the study reported that 50% reduction of epithelial cells tube formation was found reversibly by mecamylamine (10^{-7} M) and irreversibly by α-bungarotoxin (10^{-9} M);[46] (5) inhibition of nAChRs suppressed angiogenesis in response to inflammation, ischemia, and tumor growth;[6,46] (6) the angiogenic effect of nAChRs involved activation of inositol triphosphate (IP3) and diacylglycerol (DAG), protein kinase A (PKA)/MAPK, phosphoinositide-dependent protein kinase 1 (PDK1)/Akt/endothelial nitric oxide synthase (eNOS), and NF-κB.[6,39,46] The schematic signal pathway was shown in Figure 1; (7) up-regulation of vascular endothelial growth factor (VEGF) and bFGF gene expression by activation of nAChRs with low-dose nicotine administration, could enhance epithelial cell survival and new capillary network formation;[32,47] (8) another benefit of nAChRs activation in stem cell therapy is improvement of paracrine effect with increased secretion of VEGF.[47]

DISPARITY OF NICOTINE'S EFFECTS

As mentioned above, nicotine exhibited dose-dependent effects. However, in nicotine-based angiogenesis the duration of exposure or route of delivery might be an important factor. Konishi et al. showed that chronic administration of nicotine by oral route impaired nAChR-mediated angiogenesis.[48] Moreover, the augmentation of nicotine angiogenesis in ischemic limb was abolished when exposed to nicotine for 16 weeks, which was associated with reduction of plasma VEGF and vascular nAChRs expression. Park et al. also indentified decreased cell migration and tubular structure formation when HUVECs were exposed to nicotine for 2 weeks.[49]

CONCLUSIONS

Cigarette smoking is a major risk factor of CVD and nicotine was originally thought to be a key underlying offender. However, many recent studies have demonstrated that activation of nAChRs by low concentration of nicotine enhanced cell proliferation, and angiogenesis as well as anti-apoptosis. Although angiogenesis by nicotine may not all be beneficial, the physiological angiogenesis that nicotine improved, such as observed in wound healing or limb ischemia,[50] is against the fact that cigarette smoking delays wound healing. To con-
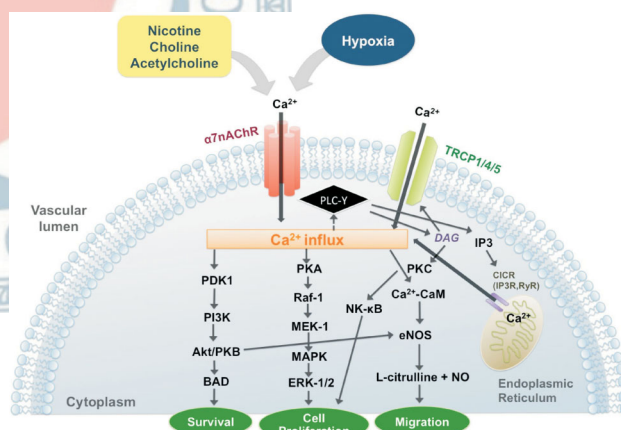

Figure 1. *The signal pathway of nicotine-induced angiogenesis via activation of nAChR in non-neuronal cells. Binding of nicotine to the α7-nAChR leads to calcium influx through ionic channel, which is enhanced by the simultaneous activation of voltage-activated Ca^{2+}-channels. The induced calcium permeability recruits phospholipase C (PLC-γ), leading to the formation of inositol triphosphate (IP3) and diacylglycerol (DAG). DAG then activates a signaling cascade through protein kinase C (PKC) and increased intracellular Ca^{2+} through (RAF1) and the mitogen-activated protein kinases (MAPK) cascade. At the same time, IP3 acts on endoplasmic reticulum (ER) and releases Ca^{2+} from intracellular stores. These signaling cascades from calcium influx lead to stimulation of cell proliferation, migration and survival.*

clude, a low concentration of nicotine delivered over a short-term period, and not administered orally, can improve angiogenesis and prevent apoptosis under hypoxia condition. There will be opportunities for further development of novel treatments and prevention in the future if follow-up research on the effects of nicotine through nAChRs continues.

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